Amendments to the Specification

Please replace paragraph 1 with the following amended paragraph.

~~This application claims priority of U.S. Provisional Application No. Serial 60/404,426, entitled "Methods for Covalently Attaching Proteins to Substrates," filed August 20, 2002, and is a continuation-in-part of U.S. Patent Application No. 09/941,833, entitled "Methods for Covalently Attaching Nucleic Acids to Substrates," filed August 30, 2001.~~ This application claims the benefit of U.S. Provisional Application No. 60/404,426, filed August 20, 2002, and is a continuation-in-part of U.S. Patent Application No. 09/941,833, filed August 30, 2001 (now abandoned). The entire contents and disclosures of which are hereby incorporated by reference.

Please replace paragraph 4 with the following amended paragraph.

Protein microarrays have been developed for high-throughput analysis of protein function (see MacBeath et al., *Science*, 289, 1760-1763, 2000). However, current technology leads to a problem of reusability. There is a need for increased uniformity and strength of attachment, which would result in more uniform fluorescent signals and decreased protein loss during the course of experiments. Also, if more stringent wash conditions were feasible, this could reduce background and thus allow for greater sensitivity. There is thus a need for increased reproducibility and consistency of results, and enhanced stability that allows the reuse of protein microarrays. None of the existing technologies provide a means to fulfill these needs.

Please replace paragraph 69 with the following amended paragraph.

Before covalently bonding a target polypeptide to a glass substrate, ATMS-reacted substrates are preferably converted to the benzene diazonium form by treatment with a solution containing HCl and $NaNO_2$ as illustrated in FIG. 2. Primary aromatic amines react with nitrous acid to yield diazonium salts. In this step, an electrophilic attack by ^{+}NO causes displacement of the H^{+} at the nitrogen (Morrison et al., eds., *Organic Chemistry*, (Third ed.) 1973):

Please replace paragraph 71 with the following amended paragraph.

Because diazonium salts are unstable, this and all subsequent steps of this process, inclusive of nucleic acid, biomolecule or polypeptide spotting, are preferably performed at 4°C. After 30 minutes, the ATMS-treated substrates are preferably washed successively with ice-cold sodium acetate buffer, double-distilled H_2O, and 100% ethanol. A gentle acidic buffer keeps the diazonium salts active (Morrison et al., eds., *Organic Chemistry,* (Third ed.) 1973) on the glass substrate.

Please replace paragraph 72 with the following amended paragraph.

In a preferred embodiment of the present invention, as illustrated in FIGS. 4 and 5, polypeptides may be spotted or microarrayed onto the diazotized glass substrate produced by the process of FIG. 3 and air-dried at room temperature for 1-2 hours. The polypeptide reacts to form a covalent bond with the azo-terminus of the diazotized substrate (Morrison et al., eds., *Organic Chemistry*, (Third ed.) 1973). In order to neutralize unreacted diazonium groups and to reduce nonspecific binding of the polypeptide to the slide, the substrates are preferably immersed in 1% glycine solution or up to 5% BSA solution.

Please replace paragraph 73 with the following amended paragraph.

Diazonium salts can undergo a reaction referred to as "coupling," in which certain aromatic compounds covalently bind to the positively-charged nitrogen of the diazonium group (Morrison et al., eds., *Organic Chemistry*, (Third ed.) 1973). In the reaction between the diazonium salt on the glass substrate and the polypeptide, any aromatic rings of the polypeptide undergo attack by the diazonium ion. Because the diazonium ion is very weakly electrophilic, the aromatic ring preferably contains a powerful electron-releasing group, *i.e.*, -OH, $-O^-$, $-NR_2$, -NHR, or $-NH_2$. Covalent binding usually occurs *para* to the activating (electron-releasing) group (Morrison et al., eds., *Organic Chemistry*, (Third ed.) 1973).

Please replace paragraph 74 with the following amended paragraph.

Although only the process for forming a diazotized glass substrate is described in detail above, the present invention can utilize a variety of substrates. With the exception of noble metal substrates and aromatic polyamide substrates (which may still contain aromatic amine monomers following polymerization and, as such, can possibly undergo diazotization directly), primary aromatic amines are generally introduced to oxidized substrates, *e.g.*, silica and plastic, by silanization, as shown above for glass. Hydroxyl groups are added to these substrates by surface oxidation reactions. For example, silicon may be treated with an oxidizing agent such as piranha solution similar to piranha solution described above for glass. Polymers, *e.g.* plastics, can be oxidized by a variety of oxidation techniques including the use of corona discharge, ozone, oxygen plasma, hydrogen peroxide, nitrous acid, alkaline hypochlorite, UV irradiation, oxidizing flame, and chromic acid (R. Ebewele, *Polymer Science and Technology*, ~~R. Ebewele,~~ 2000; Gutowski et al., *Journal of Adhesion*, 43 (1) [[(#1-2)]], 139-155, 1993; Duffy et al., *Journal of Micromech. Microeng.*, 9, 211-217, 1999; Bowden et al., *Appl. Phys. Let.*, 75(#17), 2557-2559, 1999; Fadeev et al., *Langmuir*, 14, 5586-5593, 1998; Horr et al., *Journal of Adhesion Sci. Tech.*, 11(#7), 995-1009, 1995; Abdou et al., *Syn. Metals*, 60(#2), 93-96, 1993). Gutowski *et al.*, (*Journal of Adhesion*, 43 (#1-2), 139-155, 1993) used the process of corona discharge followed by application of organo-functional silane to accomplish the silanization of polyethylene. The incorporation of surface hydroxyl groups onto the polymer substrate enables organo-silane to create the hydrogen or covalent bonds with the oxidized polymer substrate. ~~Whitesides~~ Duffy *et al.* (*Journal of Micromech. Microeng.*, 9, 211-217, 1999; Bowden et al., *Appl. Phys. Let.*, 75(#17), 2557-2559, 1999) have successfully accomplished the oxidation of different substrates (polymer, glass, silicon, silicon oxide) through oxygen plasma. Fadeev and ~~McCarthy'sresearch~~ McCarthy's research (*Langmuir*, 14, 5586-5593, 1998) shows that a 3-aminopropyltrialkoxysilanes modified polyethylene terephthalate (PET) substrate followed by hydrolysis is reactive to organosilanes and should react with the versatility of oxidized silicon wafers. Recent research also shows that silane can be used in modifying clay and ceramic (Dai et al., *Appl. Clay Sci.*, 15(1) [[(#1-2)]], 51-65, 1999; Yeh et al., *Ceramic Int.*, 21(#3), 181-186,

1995). Thus, based on the theory and results of recent researches, hydroxyl group terminated surfaces may include oxidized glass, oxidized silica substrate, and oxidized polymer. Clay and ceramic are able to be modified by silane. Glass, silicon wafer, polymers such as plastics, clay and ceramic are thus all suitable substrates for the present invention after the pre-treatment of oxidization.

Please replace paragraph 85 with the following amended paragraph.

Method for Diazotizing Glass, Plastic, and Other Substrates. Glass or silicon substrates requiring a pre-oxidation step were cleaned by immersion into piranha solution (70/30 v/v sulfuric acid and 30% hydrogen peroxide) for 30 minutes followed by washing in deionized water (Moon et al., *Langmuir*, 12, 4621-4624, 1996). Cleaned substrates were coated with ATMS by immersing the substrates in 1 mM solution of p-aminophenyl trimethoxysilane in ethanol for 30 minutes. The substrates were then rinsed in ethanol and dried in a stream of N_2. This procedure resulted in the formation of an amine-terminated layer on the substrate. Formation of silane layers was confirmed by X-ray photoelectron spectroscopy. The thickness of the ATMS layers was estimated by ellipsometry of monolayers formed on substrate-oxidized Si wafers (Moon et al., *Langmuir*, 12, 4621-4624, 1996). The ellipsometric thickness of this ATMS layer was 4.9 ± 0.2 Å.